NATIONAL CHILDREN'S STUDY
PUBERTY CORE HYPOTHESIS

I. Proposed hypothesis

Pre- and post-natal environmental factors can alter age at onset, duration, and completion of puberty.

II. Workgroup

Fertility & Early Pregnancy (Collaboration with Growth; Exposure to Chemical Agents)

III. Contact persons

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IV. Public Health Significance

Over the last century, better child-care (primarily as a result of increased food supply, improved health and sanitation services, and a higher level of education) has produced a taller stature and a more rapid maturity in Europeans, North Americans, and Australians. It is a phenomenon that has been referred to as "the secular trend in growth." Some of the supporting evidence comes from an increasing number of reports from pediatricians in industrialized countries indicating that many girls present with secondary sex characteristics at a younger age than has previously been considered normal (e.g., Papadimitriou, 2001; Herman-Giddens et al., 1997). Recent data from the NHANES III suggests that the rate of growth for boys in the U.S. is much faster than earlier born males (Karpati et al., 2002). Questions have been raised about the secular trend in growth in terms of its possible relationship to factors other than those thought to be primarily responsible. Thus, it has been proposed that the trend may also reflect the impact of one or more environmental or socioeconomic factors, and that certain ethnic, social and economic subgroups of the population may be more vulnerable than others to such factors.

The full spectrum of adverse effects produced by altering the normal human developmental process is currently unknown, but there have been many studies confirming that an abnormal pubertal process can have profound physiological, psychological and behavioral impacts on children. For example, obesity occurs at a higher rate among children with central precocious puberty (CPP, Palmert et al., 1999), and the premature pubertal growth spurt (with less time for prepubertal growth) results in reduced adult height (Schoevaart et al., 1990). Other studies have shown that certain girls with premature adrenarche are at risk of developing functional ovarian hyperandrogenism, polycystic ovarian syndrome, and hyperinsulinism (Vuguin et al., 1999; Banerjee et al., 1998). Early menarche is also reported to be a risk factor for breast cancer, underscoring the role of early developmental milestones as indicators for adult onset disease (Vihko & Apter, 1986). Psychological and psychosocial disturbances are also associated with precocious puberty; CPP often leads to lower self-esteem, and early menarche has been associated with comorbid depression and substance abuse (Stice et al, 2001). Delayed puberty

(DP) can result in short stature and lack of sexual development, characteristics which may lead to emotional and social difficulties. Bone mass gain is rapid during puberty, and recent data suggest that a delay in pubertal maturation may cause prolonged, possibly irreversible defects in bone mineralization, thus altering peak bone mass and interfering with the normal bone accretion process, later causing osteoporosis (Rakover et al., 2000; Moreira-Andres et al., 1998.)

Many factors are known to contribute to precocious puberty (PP) and DP. For example, several studies indicate that obesity is an important contributing factor (Kaplowitz et al., 2001). This is considered one of the leading causes in the United States as the incidence of obesity in children is increasing at an alarming rate. Various neoplastic and other diseases (eg., neurofibromatosis) can also contribute to PP, whilst exposure to chemical pollutants (phthalates) has been suggested as the source of multiple cases of premature thelarche in Puerto Rican girls as young as three (Colon et al., 2000). Even social factors (family stress, presence of stepfather) have been implicated (Ellis & Garber, 2000). Likewise, there are a number of factors for increased risk of DP, including chronic illness (e.g.. sickle cell disease, thalassaemia), malnutrition, and endocrine deficiencies.

It is important that we increase our ability to identify early changes in the distribution of age at onset for puberty in both girls and boys, and to elucidate any relationships with adult-onset disease. Further, population surveillance will be useful in identifying early and subtle markers of reproductive and developmental toxicity and their implications for adult health and disease.

V. Justification for a large, longitudinal study

The interindividual variation in onset and duration of puberty covers 15 years or more, and the potential impact of a wide range of environmental, socioeconomic and genetic factors, acting from conception onwards, on the normal process, and any adverse outcomes they produce, cannot be assessed without a large, longitudinal study. Sample size estimates based on existing methods for monitoring puberty and information about the timing of puberty available in the literature are appended in table form below; generally these range between 5000 and 20,000 children. These were based on estimates of exposure to relatively high levels of chemicals of concern set at 10 or 20% of the study population. Refinements in methods (see VII) would be expected to improve the power.

VI. Scientific Merit

Puberty is a key process in human development. Considering its importance, there is incomplete understanding and lack of consensus on:

(A) What constitutes normal pubertal development? Current evidence suggests that there has been a change in the distribution of age of onset of puberty, primarily towards the younger end. This may be due to some or all of the factors identified as producing the secular trend in growth. However, it may also be due to measurements of puberty being outdated, insensitive or inaccurate. For example, limited data are available on male puberty and precise markers of progression are not available (there are no objective measures in males such as menarche in

females). In general, secondary sex characteristics are considered surrogate markers despite recognition that puberty begins with primary changes in gametes. A large, longitudinal study would enable researchers to obtain samples from multiple individuals at multiple time points of development. This would permit a thorough characterization of puberty - what its full spectrum of characteristic events are (molecular, cellular, systemic and anatomical), and the normal spectrum of differences that can be expected.

The true incidence of PP and DP is somewhat nebulous. The incidence of DP in particular has historically not been well reported, and there is a particular paucity of information available on both PP and DP in boys. Thus, there is a need for epidemiological studies to clarify both the source and extent of abnormal pubertal development at the national and local population level.

Prior large studies such as NHANES have been cross-sectional - so they are actually looking at pubertal characteristics at a given age but not the onset of puberty. And NHANES is unable to effectively link earlier exposures to alterations in the timing of puberty.

(B) What are the long-term effects of delaying or elongating the pubertal process? There is little information on the impact of a shift in the pubertal period on reproductive health.

(C) What factors can cause such changes? Explanations of the secular trend in growth do not normally include the impact of environmental factors such as endocrine active compounds (EACs). This may be because only limited data are available suggesting any kind of link. These do, however, include a recent study by Colon et al. (2000), which found elevated concentrations of phthalates in the serum of girls with premature thalarche. In another study, *in utero* and lactational exposure to polybromenated biphenyls was associated with an earlier age of menarche (Blanck et al., 2000). Krstevska-Konstantinova et al. (2001) found p,p'-DDE, (a metabolite of the organochlorine pesticide, DDT) in a significant number of precociously pubescent children who had immigrated to Belgium from developing countries. These examples represent a very limited body of work carried out to assess the effects of environmental exposure to EACs in human pubertal development, and there is clearly a need to confirm and carry out further studies of this kind. Numerous children living in various communities are exposed on a daily or seasonal basis to relatively high concentrations of pesticides and other chemical classes, many of which are known to be EACs in lab animals. There is a need both to develop methods to accurately and conveniently measure the exposure levels, and to perform health outcome studies to assess the impact, if any, of such exposures on pubertal development. We would not be the first to call for such studies. Partch and Sipplle (2001) recently suggested that studies of the effects of defined environmental oestrogenic substances on the human reproductive system and on pubertal development are warranted. If such effects are apparent, it is important to distinguish if they are initiating secondary sexual characteristics (peripheral puberty, PP) or central puberty (true puberty: spermatogenesis, folliculogenesis).

Explanations of the secular trend in growth do not normally include the impact of socioeconomic factors. Clearly those populations with less privileged lifestyles and in poorer socioeconomic communities are more often more vulnerable to social and environmental injustice and nutritional and adverse environmental exposures. The make-up of family units is more varied

today than ever before, and it is widely acknowledged that children are subject to more stress than any generation in history (one only has to look at the recent increase in child and adolescent suicide rates). The impact of stressful sociologic factors has been related to precocious pubertal development. Furthermore, there is need to examine the widespread social implications of the secular trend of early growth. If indeed there has been a shift in the populations towards sexually development at an earlier age, what consequences will this have on the average age at which sexual activity is first engaged in, and how will this impact the psychological status of the young mothers, the individuals and society which must sustain them, and the development of the infants themselves.

VII: Potential for innovative research

(1) This research will refine current tests for puberty and the development of more objective and sensitive indicators, including biochemical and molecular biomarkers. For example, the evaluation of sperm in urine (spermaturia) has been used previously to assess the age of onset of spermarche. It has been shown that spermatogenesis can begin before any other signs of puberty (Nysom et al., 1994). This conclusion was obtained from spermaturia studies in two normal boys with testicular volumes of 3 ml and no other signs of puberty, and indicates that the definition of start of puberty as testicular volumes of 4 ml or more may be too rigorous. Spermaturia is a more common and regular event during early and mid-puberty than in more mature subjects, but one problem preventing its regular use as an indicator of puberty is the intermittent occurrence of sperm-negative urine samples. The incorporation of spermaturia as a test for puberty in a large sample group would give this test more power to determine the distribution of the age of onset. Another possible target for investigation is antisperm autoantibodies, which have been shown to appear after sexual maturation in rats (Flickenger et al., 1997). The animals underwent normal pubertal development, and the rise in antisperm antibodies correlated temporally with events in the postnatal development of the male reproductive system. Mullerian inhibiting substance (MIS) is an example of a serum protein biomarker that might be used to evaluate pubertal onset. MIS values for males rise rapidly during the first year of life (uniformly measurable in all prepubertal boys) and are highest during late infancy, then gradually decline until puberty (Lee et al., 1996). MIS levels correlate better with developmental age than chronological age, and males with delayed puberty have elevated levels. In contrast, MIS is undetectable in most prepubertal female subjects. Similarly, the gonadal hormone inhibin B has been found to be highly expressed in infant boys and decreases gradually to a nadir at 6-10 years of age (Crofton et al., 2002). Brugo-Olmedo et al. (2001) suggested that serum inhibin B may be a reliable marker of the presence of testicular spermatozoa in patients with nonobstructive azoospermia. If this is confirmed, it could be used as another biomonitoring approach to assessing pubertal onset in boys. Inhibin A and B, activin A and follistatin are found at different levels in girls depending on pubertal stage (Foster et al., 2000), suggesting that significant changes in serum concentrations of these and other FSH-regulatory peptides accompany the onset and progress of puberty, and should be investigated as possible alternative staging markers for pubertal development.

(2) Monitoring puberty in a large number of individuals through out childhood and adolescence

would provide normative data across groups on the timing of puberty and allow identification of a broad spectrum of adverse effects associated with altered onset.

(3) Developing new techniques for monitoring children's health, such as the use of surrogate tissue analysis (STA) to monitor events in inaccessible target tissues using accessible biological samples such as blood, hair follicles and urine.

VIII. Feasibility

Critical period for exposure and outcomes: Limited data are available on specific critical windows. The number of factors that can impact puberty is quite high and the entire prepuberal period, including *in utero* growth and development, should be considered as a critical period. The outcomes being measured are pubertal landmarks and biomarkers (some yet to be determined) that indicate one or more pubertal landmarks. Since precocious puberty can start as young as 3 (exceptionally, it has been seen at 12 months), it may be prudent to begin collect samples as soon as possible after birth.

Sampling needs: Potentially all children in the National Children's Study. Subgroups of special interest include those in rural communities exposed chronically or seasonally to pesticides; African-American girls (who usually demonstrate an earlier entrance into puberty than other races, implying unique genetic factors); children with certain diseases or conditions (For PP - neurofibromatosis, hypothyroidism, polycystic ovary syndrome, etc; DP - sickle cell disease, thalassaemia, Celiac disease, Gaucher disease type 1, Cushing's disease, etc.); populations consuming foods such as fish with high concentrations of bioaccumulative EACs; sampling could include dietary intake, food diaries, residential and work samples.

Contact*:* Clinician examination during routine exams until age 6 for girls and 7 for boys, followed by routine examination and home collection of data. Precise intervals will be determined during assessment of methods.

Nature of measurement: Interview, observation (Tanner scales), questionnaires. Physical samples could include breast milk for analysis of pesticides, etc.; urine for steroid metabolites, sperm and RNA (uroepithelial cells); blood/serum for hormone measures (e.g. MIS, inhibin, sex steroids,) and RNA (immune cells); hair for drug and heavy metal exposure; hair follicles for RNA; buccal cells for DNA analysis (for polymorphism and disease alleles).

Burden on participant and family: Initially minimal (done during routine exams) with modest burden after age 6 for girls and 7 for boys. This would include frequent urine sampling (monthly/quarterly).

Ethical considerations: 1. Embarrassment about pubertal issues; 2. There is a possibility that samples might be screened for certain disease alleles. Appropriate targets would need to be selected; 3. Some minimally invasive procedures possible; 4. Right to privacy; 5. Consequence of adverse findings; 6. Provision of medical care upon discovery of adverse findings; 7. Payment of medical services for participants who belong to health organizations (standard v non-standard).

Girls

Total sample size required (α=0.05, 1-β=80%, exposed group 1 year EARLY/LATE mean age at onset, 5% loss to follow-up)

Breast Development

Race	Reference	Not Exposed Mean Age	Exposed			
			10%		20%	
			EARLY	LATE	EARLY	LATE
African American	W	9.50	9,376	7,767	5,593	4,615
	HG	8.87	8,229	6,722	4,906	3,991
White	W	10.50	11,364	9,582	6,775	5,695
	HG	9.96	8,581	10,264	5,095	6,121
Mexican American	W	10.30	9,208	10,946	5,467	6,529

Pubic Hair

Race	Reference	Not Exposed Mean Age	Exposed			
			10%		20%	
			EARLY	LATE	EARLY	LATE
African American	W	9.50	9,376	7,767	5,593	4,615
	HG	8.78	6,579	8,064	3,907	4,813
White	W	10.30	9,208	10,946	5,467	6,529
	HG	10.51	9,604	11,386	5,704	6,787
Mexican American	W	9.80	8,295	9,956	4,927	5,935

Menses

Race	Reference	Not Exposed Mean Age	Exposed			
			10%		20%	
			EARLY	LATE	EARLY	LATE
African American	W	12.10	12,882	14,928	7,652	8,899
	HG	12.16	13,014	15,071	7,735	8,983
White	W	12.70	14,246	16,391	8,462	9,771
	HG	12.88	14,664	16,853	8,713	10,039
Mexican American	W	12.20	13,102	15,170	7,785	9,043

Boys

Total sample size required (α=0.05, 1-β=80%, exposed group 1 year early/late mean age at onset, 5% lost to follow-up)

Increased Testis Size

Race	Genital Stage	Reference	Not Exposed Mean Age	Exposed			
				10%		20%	
				EARLY	LATE	EARLY	LATE
All	2	K - NH	9.9	8,471	10,154	5,031	6,050
	3	K - NH	12.2	13,102	15,170	7,785	9,043
	3	K - HES	13.0	14,950	17,150	8,884	10,225
	4	K - NH	13.8	16,919	19,262	10,057	11,475
	4	K - HES	13.9	17,172	19,526	10,207	11,635
	5	K - NH	15.8	22,375	25,048	13,297	14,923
	5	K - HES	15.1	20,384	22,936	12,109	13,665
White	2	HG	10.1	8,834	10,550	5,246	6,289
African American	2	HG	9.5	7,767	9,376	4,615	5,593
Mexican American	2	HG	10.4	9,395	11,155	5,581	6,649

Pubic or Auxillary Hair Growth

Race	Genital Stage	Reference	Not Exposed Mean Age	Exposed			
				10%		20%	
				EARLY	LATE	EARLY	LATE
All	2	K - NH	11.9	12,442	14,455	7,393	8,617
	3	K - NH	12.6	14,015	16,149	8,325	9,625
	3	K - HES	13.3	15,676	17,931	9,313	10,687
	4	K - NH	13.6	16,413	18,723	9,757	11,155
	4	K - HES	14.0	17,436	19,801	10,357	11,801
	5	K - NH	15.7	22,085	24,740	13,123	14,743
	5	K - HES	15.3	20,943	23,530	12,445	14,019
White	2	HG	12.0	12,662	14,697	7,525	8,761

African American	2	HG	11.2	10,968	12,860	6,517	7,669
Mexican American	2	HG	12.3	13,333	15,412	7,921	9,187

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